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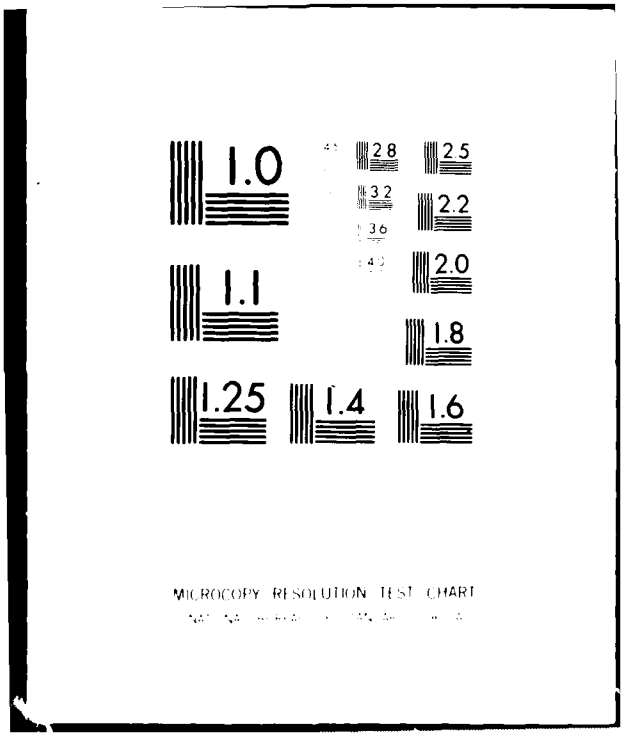
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EFFECTS OF LONG-TERM IMMUNIZATION WITH MULTIPLE ANTIGENS

FINAL REPORT

by

COMMITTEE ON THE EFFECTS OF MULTIPLE IMMUNIZATIONS
NATIONAL RESEARCH COUNCIL

JANUARY 1980

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A study of the literature on the long-term effects of multiple immunization over a prolonged period. Except for a small (approximately 99 persons) follow-up of Fort Detrick personnel, studies were not directly pertinent to the question. Recommendations are made for newer screening studies which might be undertaken on larger groups to resolve this question.

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NOTICE

The project that is the subject of this report was approved by the Governing Board of the National Research Council, whose members are drawn from the Councils of the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine. The members of the committee responsible for the report were chosen for their special competences and with regard for appropriate balance.

This report has been reviewed by a group other than the authors according to procedures approved by a Report Review Committee consisting of members of the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine.

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PREFACE

As part of a program of occupational safety at the U.S. Army Biological Laboratory at Fort Detrick, Maryland, it had been the practice to immunize workers repeatedly with multiple antigens of infectious agents to which they might be exposed. At the same time, there has been concern that such immunizations might lead to adverse effects; similar concern is felt about a possible hazard of multiple immunizations in other situations.

In 1956, a study was initiated at Fort Detrick with a group of 99 white men who were intensively studied for adverse effects of multiple immunizations. In 1971, 76 members of the original group were studied; no findings indicative of neoplastic, immunologic, or other long-term consequences of multiple immunizations were detected.

The U.S. Army Medical Research and Development Command asked the National Research Council (NRC) to examine the data available on repeated multiple immunizations, to state whether conclusions can be drawn about adverse effects of such immunizations, and to recommend tests and procedures for future studies of intensively immunized persons. This report, by the Committee on the Effects of Multiple Immunizations, established in the NRC's Assembly of Life Sciences, is a response to that request.

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INTRODUCTION

Although much success has been achieved in inducing human resistance to various infectious microorganisms or their toxic products, immunization programs are still subject to some risks.

The efficacy of a vaccine requires careful evaluation, so that efficacy can be weighed against safety with respect to immediate and delayed reactions. Assessment of the importance of early febrile, toxic, and local reactions and of anaphylactic potential is essential. Other possible adverse reactions may result--e.g., abnormal interaction between antigen and antibody may give rise to allergic encephalitis, orchitis, thyroiditis, or other abnormal conditions such as Arthus's reaction or a hypersensitivity reaction. Disorders that are ordinarily nonmalignant may be abnormal immunologic reactions; after an immunization procedure, other disorders, such as the Guillain-Barre syndrome, may occur by a mechanism not yet clarified. The administration of vaccines containing viable agents can result in excessive proliferation of the inoculated organisms in the host or a reversion to virulence or to neurovirulent or oncogenic factors; such vaccines have the potential for spread to others. Immunization may pose special risks to patients with inherited or acquired immunodeficiency states.

The possibility of delayed or unanticipated long-term risk associated with multiple immunization procedures is an acknowledged concern and requires continued scrutiny. The greatest potential risk may be in persons who receive multiple doses of vaccines that contain different

antigens over long periods. Amyloidosis, hypergammaglobulinemia, and multiple myeloma can be produced in experimental animals by repeated inoculation with large doses of antigens.[58] White et al [58] have cited monoclonal gammopathy in two laboratory workers who were inoculated repeatedly with cholera vaccine.

Various groups have traditionally received repeated immunizations, such as workers in microbiology laboratories and other health facilities, members of the armed forces, international travelers, and patients undergoing desensitization programs in allergy clinics. Limited long-term followup information is available on such groups, although some reports have described followup studies after desensitization programs,[35,61] repeated use of single antigens,[31,58] or military immunizations.[5]

A group of 99 white men, microbiologists who worked at the now-discontinued U.S. Army Biological Laboratory at Fort Detrick, Maryland, were repeatedly and intensively immunized. In 1956, they were selected for inclusion in a prospective study to determine whether long-term risks were associated with human "hyperimmunization." Results of these analyses were submitted in 1956,[46] 1962,[47] and 1971-1972.[58] The latest survey provided a followup on all but two members of the original 99. In comparison with age-matched controls (Fort Detrick employees who did not participate in the immunization program), the study group showed a persistent slight increase in serum hexosamine concentration, decreases in serum iron and albumin concentrations, a slight increase in serum copper concentration, and a slight increase in red-cell sedimentation rate. These differences were found to be statistically significant,

although their biologic significance was uncertain.[58] In this small group, there was no increase in deaths or in detectable lymphatic-system or malignant disorders. Nevertheless, the possibility of a medical disorder in a "hyperimmunized" subject merits further evaluation and continued surveillance. An ad hoc group of immunoepidemiologists of the National Cancer Institute (NCI) is determining the feasibility of locating a substantial fraction of the larger group of repeatedly immunized civilian employees at Fort Detrick.

The U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) laboratories at Fort Detrick used eight different experimental vaccines and toxoids in approximately 3,500 military and civilian laboratory personnel who received booster inoculations after multiple antigens had been given over extended periods. They were evaluated periodically for specific antibody titers, delayed dermal hypersensitivity, and lymphocyte responses in some. The present Committee has been asked to suggest studies for evaluation of potential risks associated with prolonged intensive immunization programs, such as the one at USAMRIID and those which will involve U.S. military forces elsewhere.

Generally, the U.S. armed forces initiate a multiantigen immunization series in each new inductee and maintain a program of periodic booster inoculation with other vaccines, depending on the duties and geographic areas to which inductees are assigned. Immunization requirements are continually evaluated by preventive-medicine officers of the individual services and by the Armed Forces Epidemiological Board, which

serves in an advisory capacity. Similarly, the Public Health Service Advisory Committee on Immunization Practices regularly updates its recommendations for vaccine use in civilians.[55]

The National Research Council (NRC) was asked to evaluate the real and potential long-term risks of giving multiple biologic vaccines repeatedly to adults. The immunization regimens currently used to protect public-health and hospital laboratory personnel who work with a variety of highly virulent microorganisms were to be emphasized. It is recognized that selected groups of military personnel generally receive less frequent and smaller quantities of antigens than laboratory workers. The Committee on the Effects of Multiple Immunizations was convened in the NRC Assembly of Life Sciences to conduct an evaluation of the long-term risks of multiple immunizations in adults. This report is based on a review of all available data that might bear on the problem of repeated multiple immunizations and on reinterpretation and analysis of such data in the light of current immunologic concepts. Suggestions are made as to how to obtain additional data on potential risks.

The Committee has conducted a review of the available literature in relation to the adverse effects of multiple long-term immunizations. Because reliable data are so sparse, it has also formulated guidelines and recommendations with respect to feasible and helpful measurements that can be made in future prospective studies and analyses.

If the USAMRIID desires further evaluation of this subject after completion of the proposed studies by NCI's epidemiologic unit, a request should be addressed to the National Research Council.

LITERATURE REVIEW

The MEDLARS system of the National Library of Medicine was used to search the medical literature for reports on the topic of reactions to multiple immunizations. Of the eight papers on human reactions that were found, two had some relevance to the Committee's assignment. All eight are summarized below and in Table 1, because they may reflect current frequency and medical awareness of vaccine reactions.

In the two retrospective studies, Nilsson and Widström[41] described a lack of association of BCG vaccination of young children with a subsequent increase or decrease in the occurrence of malignant tumors, and Fukuyama et al [19] found no significant relationship of immunizations with later onset of infantile spasms.

In the studies of group responses to immunization, Myers et al [40] showed that antibody response to influenza vaccine in patients with multiple sclerosis is equivalent to that in the general population (reactions were insignificant), and Pandey et al [44] opened a promising new field of study with the demonstration that, among several antigens administered to white and black infants, great responses to two, Haemophilus influenzae and meningococcus type C polysaccharides in white infants, were associated with a particular immunoglobulin allotype. The studies of White et al [58] also showed no significant long-term effects of vaccines at Fort Detrick.

TABLE 1

Summary of Literature Search for Complications of Immunizations

Immunizing Agent	Type of Complication	Age and Sex	Time of Onset after Immunization	Comment	Reference
Retrospective Studies:					
1. BCG vaccination	None	Children	Review covered several years after vaccination	Retrospective study of BCG vaccination and malignant tumors; no relationship.	Nilsson and Widström[41]
2. Diphtheria, pertusis, tetanus in various combinations; also a few smallpox and Japanese B encephalitis (110-case study)	Infantile spasms (this was a study of immunization complications in patients with infantile spasms)	Infants	One month selected for study	No more than 4.8% of patients could have had their disease result from vaccines (relationship doubtful)	Fukuyama et al [19]
Group Responses to Immunization:					
3. Influenza A/NJ/1976, influenza A/Victoria/75; 200 chick-cell agglutinating units each, inactivated; prospective study in patients with multiple sclerosis	None	Adults, male and female	--	Vaccine was safe and effective in multiple sclerosis patients	Myers et al [40]

Table 1 - continued

Immunizing Agent	Type of Complication	Age and Sex	Time of Onset after Immunization	Comment	Reference
4. *Haemophilus influenzae* and meningococcus polysaccharides	None	Infants	None	A study in black and white children of association of response to immunization and immunoglobulin allotypes; Km(1) allotype and response to *H. influenzae* and meningococcus C polysaccharides were associated in white children	Pandey *et al* [44]
Case Reports:					
5. Tetanus toxoid	Acute meningo-encephalitis, non-infectious	Adult, male	A few hours	Severe reaction	Britton *et al* [10]
6. Tetanus, diphtheria, and oral polio	Transverse myelitis	7 mo., female	6-7 days	Bilateral partial paralysis, lower extremities; polio virus type 2 recovered from stools	Whittle, and Roberton [59]
7. Typhoid A and B and cholera (subcutaneous route)	Acute renal failure, hepatitis	21 yr, male	1 hr	Recovery in a few days	Eisinger and Smith [18]
8. Rabies Semple vaccine, 14 injections	Progressive degeneration; CNS disease	24 yr, male	4 yr	Multiple sclerosis-like disease after administration of antigen derived from nervous tissue	Britton *et al* [10]

The other four papers were case reports. Three described acute reactions (two neurologic and one renal) to commonly used vaccines, and the fourth reported a case of progressive central nervous system degeneration that began 4 yr after administration of rabies vaccine derived from nerve tissue.

These few studies indicate that serious reactions to vaccines, acute and chronic, are infrequent, not reported, or not recognized. Although some reactions will escape detection or reporting, there is no reason to think that vaccine reactions constitute a large problem in the general population. Obviously, it will always be necessary to weigh the risks of vaccines against their benefits.

DISEASES OCCASIONALLY ASSOCIATED WITH MULTIPLE IMMUNIZATIONS

This section reviews some of the most important experimental studies concerning disease states that may be associated with multiple immunizations and the evidence that such diseases occur in man.

SERUM SICKNESS

STUDIES IN ANIMALS

Repeated administration of antigen is a well-established method of producing serum-sickness glomerulonephritis and renal or extrarenal vasculitis in experimental animals.[16]

Experimental acute serum sickness is usually induced by a single injection of a large amount of foreign protein. The incidence of the lesions is variable and depends mainly on the antibody response. The renal glomeruli and the vessels of the heart, the spleen, the lung, and the intestine are the tissues most frequently involved. The glomerulonephritis is characterized by proliferation of glomerular cells and accumulation of neutrophils in capillary lumina, which are partially or completely obstructed. The lesions are irregularly distributed and heal rapidly with no traces of sclerosis. Transient proteinuria, microhematuria, and oliguria are observed in most affected animals. Immunofluorescence study shows fine granular deposits of immunoglobulins, complement, and, more rarely, antigen (presumably in the form of immune complexes) along glomerular capillary walls and in the mesangium. By electron microscopy, opaque aggregates of foreign material, corresponding to the granular immunofluorescent deposits, are seen on the epithelial side of the glomerular basement membrane.

In medium-sized muscular arteries in several organs, infiltration of inflammatory cells and fibrinoid necrosis are found. In acute serum sickness, antigen-antibody complexes formed in the circulation activate several host mediators, including platelets, and release factors that increase vascular permeability. Polymorphonuclear leukocytes, complement, and fibrinogen products may be involved in the pathogenesis of the lesions. The immune complexes have no immunologic relationship to the tissue that they damage in serum sickness. The factors that predispose to their localization are related mainly to the anatomic structure and physiologic properties of the vasculature.

Experimental chronic serum sickness may be induced in experimental animals by repeated injections of an antigen over a long period.[17] The animals develop proteinuria, hypoproteinemia, and increased serum cholesterol and urea. The most common lesion is membranous glomerulonephritis characterized by thickening of capillary walls, with diffuse electron-opaque deposits of foreign material on the epithelial side of the basement membrane, corresponding to granular deposits of antigen-antibody and complement, which can be seen by immunofluorescence study. As the disease progresses, proliferation of mesangial cells and sclerosis become more evident. The most important pathogenetic factors are the quantity and the quality of the immune response. Animals generating large amounts of precipitating antibodies may develop rapidly resolving acute glomerulonephritis in the first week. Then, as soon as the animals have made sufficient antibodies to achieve permanent antibody excess, the antigen is quickly removed from the circulation as insoluble immune complexes. Most of these animals do

not develop progressive glomerulonephritis. Likewise, the animals that do not generate a sufficient amount of antibody do not develop renal disease. In contrast, the animals that form too little antibody to cause precipitation of the antigen, but enough to result in formation of soluble immune complex that persists in the circulation for a long time, develop membranous glomerulonephritis. Most of these animals generate low-affinity antibody. Complement factors participate in the production of injury.

STUDIES IN MAN

The extensive work partially summarized above provided the experimental evidence that multiple injections of antigen carry the potential risk of immune-complex formation and vasculitis of the serum-sickness type. The recent development of methods for detecting and measuring antigen-antibody complexes in biologic fluids[33] and the systematic immunopathologic studies of human tissues obtained by biopsy convincingly show that formation of immune complexes in the circulation or in tissue is frequently associated with inflammatory injury.[13] There is little evidence, however, that multiple immunizations are a common cause of immune-complex disease in man. The reports published before 1950 are rather anecdotal and devoid of reliable documentation.

In 1965, Peeler *et al*[47] described the results of clinical and laboratory investigations on a group of 99 workers, ranging in age from 28 to 65 yr, who had undergone intensive immunization to a variety of bacterial, rickettsial, and viral agents over a period of 8-13 yr. During this time, they had received an average total of over 50 ml of the various vaccines. They developed no clinical illness that could

be attributed to multiple immunizations. However, excluding persons with a history of hypertension, vascular diseases, and glomerulonephritis (whose etiology and pathogenesis were undetermined and thus not necessarily unrelated to multiple immunizations), 15 persons had 2+ proteinuria in 1956 and eight had 1+ proteinuria in 1962. More precise data were not provided.

In 1958, Stefanini et al [54] described a case of acute vascular purpura that followed immunization with Asian influenza vaccine. The patient's urine contained increased amounts of protein and red blood cells, and the plasma creatinine rose to 2.4 mg/dl. The course was benign; a few days later, the urine was no longer abnormal.

A study published in 1966 by Baylon and Bernard (cited in Wilson[60]) reviewed the incidence of nephropathy in the French Army after vaccinations administered in 1952-1964. Most of the cases were related to the introduction of a triple vaccine against diphtheria, tetanus, and typhoid-paratyphoid bacilli. The estimated incidence of nephropathy was about 1 in 1,000 vaccinations. According to the authors, many cases were missed through lack of systematic urinary controls. Two characteristic forms of nephritis were recognized. One was associated with early and sudden onset, fever, shivering, occasional circulatory collapse, hematuria, and proteinuria; the clinical course was generally benign, but relapses followed further vaccinations. The second developed late after administration of vaccine, with insidious onset, edema, increased blood pressure, azotemia, proteinuria, and microscopic hematuria. Some patients ultimately developed chronic renal failure. It should be noted that,

because the reports by Peeler et al and Baylon and Bernard failed to provide precise serologic and immunopathologic data, an exact assessment of their value is difficult.

In 1966, Bishop et al [7] described diffuse vasculitis and glomerulonephritis, similar to periarteritis nodosa, in a 45-yr-old man who had volunteered as a subject for production of hyperimmune pertussis globulin. He received one injection with 0.5 ml of tetanus toxoid and eight immunizations with 0.3 ml of pertussis vaccine containing 8 NIH pertussis protection units per milliliter of phase I killed Bordetella pertussis organisms. No adjuvant was used. During the period of immunizations, the patient was subjected to weekly plasmapheresis 23 times; each time, 1,000 ml of whole blood was removed, and the packed red blood cells were reinfused. A week after the last injection he developed hematuria, proteinuria, hypertension, and hemoptysis. The findings in tissue obtained by renal biopsy were consistent with those of subacute glomerulonephritis. His course was marked by progressive renal failure. At autopsy, there was disseminated vasculitis, with involvement of the kidneys, lungs, heart, gastrointestinal tract, testes, and adrenals. Immunofluorescence studies were not performed.

Acute glomerulonephritis in a 53-yr-old man was described in 1972 by Joekes et al.[26] Four days after administration of typhoid vaccine, he developed oliguria and azotemia, and he died 68 days later, despite hemodialysis. A renal biopsy performed on the fourteenth day of the illness showed poliferative glomerulonephritis and vascular necrosis, with granular deposits of IgG, IgM, and C_3 in the vascular walls,

but not in glomeruli. Necrotizing arteritis, compatible with a diagnosis of periarteritis nodosa, was present in the lungs and spleen.

The most recent documented case of glomerulonephritis associated with multiple immunizations was reported by Boulton-Jones et al in 1974.[9] In December 1969, a 33-yr-old nurse with features of hysterical and depressive illness was hospitalized with fever, indurated red lesions around the right knee and the forearms, hypocomplementemia, cryoglobulinemia, proteinuria, hematuria, and impaired renal function. A renal biopsy showed a diffuse membranoproliferative glomerulonephritis with C_3, but not immunoglobulin, deposits. Occasional subendothelial deposits were observed by electron microscopy. She was treated with immunosuppressive drugs and anticoagulants. By March 1972, her general condition had deteriorated. A second renal biopsy showed classic immunopathologic features of immune-complex glomerulonephritis, with proliferative and exudative lesions; granular deposits of C_3, IgG, and IgA; and diffuse electron-dense deposits. It was then determined that she had given herself injections of 2 ml of diphtheria, pertussis, and tetanus vaccine every 2 months over a period of 4 yr. Additional immunopathologic studies were performed. Antibody titers to diphtheria and pertussis were very high (1/256,000), comparable with those in hyperimmunized animals. The cryoglobulin contained IgG, IgM, and C_3. Specific uptake of radiolabeled diphtheria toxoids could not be shown in cryostat sections of the renal-biopsy tissue. When the self-administration of vaccine was interrupted, the renal function gradually improved, although sclerotic lesions had developed

in several glomeruli. It was established that the red raised lesions observed around the knee and the forearms had developed at the injection sites and thus were similar to the Arthus phenomenon. The detailed history of this patient shows striking similarities with chronic serum sickness experimentally induced in animals. There are discrepancies, especially with respect to the amount and frequency of antigen administration. In rabbits weighing 2-3 kg, daily injections of 0.5-50 mg of bovine serum albumin containing 0.08 mg of nitrogen are used.[17] In contrast, in this 55-kg patient, injections containing about 20 mg of nitrogen given every 2 months were sufficient to induce glomerulonephritis. It should also be noted that in the experimental model of Dixon et al [17] glomerulonephritis is more likely to occur with regimens generating immune complexes in moderate antigen excess, whereas the patient reported by Boulton-Jones et al was constantly in a condition of antibody excess; this confirms the view that differences in various antigen-antibody systems may be important in the genesis of immune-complex nephritis.[20] It is also possible that the pertussis vaccine induced an adjuvant effect,[28] with production of antibodies to exogenous or endogenous antigens unrelated to the vaccine, thus leading to immune-complex disease.

AUTOIMMUNE DISEASES

STUDIES IN ANIMALS

There is convincing evidence that chronic antigenic stimulation may be associated with autoimmune diseases in genetically predisposed

animals. Several mouse[2] and dog[36] strains spontaneously develop a lupus-like syndrome. Among the postulated etiologic factors are chronic retrovirus antigenic stimulation, thymic atrophy, polyclonal B-cell activation, and deficiency in suppressor T cells and other subsets of T cells. These animals have autoantibodies, circulating immune complexes, and abnormalities of IgG and complement. The organ most frequently effected is the kidney, with immune-complex glomerulonephritis and retroviral gp 70 and DNA glomerular deposits.[2]

Autoimmune lesions have been induced in animals immunized with bacterial antigens by cross-reactions of antibodies to bacterial components with tissue components. Rabbits inoculated with group A streptococci develop antibodies that bind to cardiac and skeletal muscles.[27,56] The role of these antibodies in tissue pathology is a matter of debate. Antibodies to fragments of streptococcal cell membrane cross-react with glomerular basement membrane in vitro.[37] However, there is no evidence that such cross-reacting antibodies induce glomerulonephritis in man or in animals.

Rabbits hyperimmunized with streptococci, but not with gram-negative bacteria, develop high concentrations of 19S anti-IgG; this suggests a specific role for streptococcal antigens in the induction of rheumatoid factor anti-IgG antibody.[8] Bacterial lipopolysaccharides seem to induce a polyclonal B-cell activation in the presence of a variety of antigens, including DNA,[25] and this could result in autoimmune injury. Another possible consequence of multiple immunizations

is the development of autologous anti-idiotypic antibodies,[29] which might cause immune suppression in mice.[4,15]

An analysis of possible autoimmune reactions after multiple immunizations in man should consider the specific antigenic activity of central nervous tissue. In the 1920s and 1930s, several workers showed that monkeys given repeated injections of brain extracts or emulsions over a period of several months developed acute disseminated encephalomyelitis. The use of Freund-type adjuvants during the 1940s led to the discovery of experimental allergic encephalomyelitis (as it is known today). Monkeys, guinea pigs, and rabbits develop an accelerated form of disseminated encephalomyelitis after injection of brain or spinal-cord homogenates combined with the immunologic adjuvant.[45]

Experimental allergic neuritis is induced by immunization of animals with peripheral nervous tissue.[57] The lesions are limited to the peripheral nervous tissue. This disorder is similar to the demyelinating syndrome that follows rabies immunization and some viral infections (Landry-Guillain-Barré-Strohl syndrome).

STUDIES IN MAN

Several studies have indicated that streptococcal antigens may induce formation of antibodies that cross-react with cardiac or neuronal constituents. The etiology of poststreptococcal rheumatic carditis is unknown. Various hypotheses (reviewed by McCarty[38]) include a direct effect of streptococcal product on tissues, the reaction of poststreptococcal antibodies with streptococcal antigens bound to

tissues, and the cross-reaction of antistreptococcal antibodies, or lymphocytes, with tissue antigens. About 50% of children with rheumatic chorea develop anti-Group A streptococcal membrane antibodies that cross-react with the cytoplasm of subthalamic and caudate nuclei; the presence of antineuronal antibodies appears to be correlated with the severity and the duration of clinical attacks.[24] Cytomegaloviral infection has been linked with some forms of Coombs-positive hemolytic anemias. It appears that active infection with this and perhaps other occult viruses may trigger an antierythrocyte response in some persons.[62]

Another recent report appeared to establish a relationship between viral infection and autoimmunity and suggested that EB virus activates rheumatoid arthritis or is perhaps an etiologic agent. Possibly, the virus promoted a polyclonal B-cell activation.[1]

The idea that immunizations with nervous tissue may have an injurious effect on the nervous system can be traced to the occurrence of "paralytic accidents" in man after injections of Pasteur rabies vaccine during the 1880s and 1890s. This was an attenuated-virus vaccine consisting of rabbit central nervous tissue that contained "fixed" rabies virus. Occurrence of the paralysis after injections of rabbit cord or brain that contained killed (phenol-treated) rabies virus indicated that the paralytogenic activity of the vaccine was a property of the nervous tissue, and not of the rabies virus.

Acute polyneuritis (Landry-Guillain-Barré syndrome) may occasionally follow immunization.[23,32,59] If the disorder is fatal within a

few days, perivascular lymphocytic infiltrates are found. If the illness progresses for weeks or months, inflammatory cellular infiltrates and perivenous demyelinization are combined with sequential demyelinization and wallerian degeneration. Inflammation of nerve roots evidently accounts for the increase in protein and in the number of lymphocytes in cerebrospinal fluid. Lymphocytic infiltrates are occasionally found in liver, spleen, lymph nodes, heart, and other organs and reflect the systemic nature of the disease.[3] From a pathogenetic standpoint, most of the evidence suggests that the manifestations of this disorder result mainly from a cell-mediated immunologic reaction directed at peripheral nervous tissue. However, the observation that Landry-Guillain-Barré syndrome may be associated with immune-complex glomerulonephritis[6,49,51] indicates that cell-mediated hypersensitivity is not the sole mechanism in the pathogenesis of this disease. Unfortunately, elution studies were not performed, so there is no information concerning the specificity of the immune-complex reaction. Two reports of patients with Landry-Guillain-Barré syndrome and immune-complex glomerulonedphritis suggest cellular sensitivity to glomerular basement membrane, nerve, and renal tissue antigens.[6,49] It is therefore possible that both humoral and cellular mechanisms are involved in the pathogenesis of Landry-Guillain-Barré syndrome.

PLASMA-CELL DYSCRASIA (PARAPROTEINOSIS)--MYELOMA, AMYLOIDOSIS, WALDENSTRÖM'S MACROGLOBULINEMIA

STUDIES IN ANIMALS

One of the most persistent concepts in the pathogenesis of amyloidosis is that of the association of amyloidosis with prolonged immunologic stimulation and hypergammaglobulinemia. Krawkow showed that amyloidosis can occasionally be induced in experimental animals by multiple immunizations with live or dead bacteria.[30] Shortly thereafter, it was noted that horses repeatedly immunized for production of diphtheria antitoxin exhibit a high incidence of amyloidosis. Later studies by many workers have shown that amyloid can be induced in experimental animals by multiple injections of Freund's adjuvant.[12] Minks spontaneously develop Aleutian disease, characterized by systemic plasma-cell proliferation, hypergammaglobulinemia, necrotizing vasculitis, and occasionally amyloidosis.[21] The disease, which is a viral infection, combines many of the serologic, cellular, and tissue changes of human myeloma.[50] However, the precise role of chronic antigenic stimulation in the pathogenesis of this myeloma-like disease is still unknown.

STUDIES IN MAN

Persistent antigenic stimulation could initiate a process in which a given clone of immunologically competent cells proliferates and with its progeny preferentially synthesizes antibodies directed to the specific antigenic determinants.[48] However, only very few reports of patients with myeloma and macroglobulinemia occurring after prolonged antigenic stimuation have been published. One of the patients had a lifelong history of leishmaniasis.[39]

LYMPHOMAS

The role of stimulation of the reticuloendothelial system in the genesis of reticular neoplasm has been suggested in animals[43,52] and humans.[42] Viral antigens are the most probable candidates for this stimulation. The relationship between EB virus infection and Burkitt's lymphoma is based on the consistent demonstration of high titers of anti-EB antibody in the serum of both African and American patients with Burkitt's lymphoma[11,34] and the demonstration of EB virus in cultures of cells from many patients with this form of neoplasm. Although the etiologic role of EB virus in Burkitt's lymphoma is not conclusive,[53] it seems probable that the same virus can cause malignant and benign diseases in different populations.[22] Animal experiments performed with xenotropic viruses that affect man have suggested that attenuated viruses may have oncogenic potential, and there might be a 20-yr gap before clinical signs appear. However, there is no reliable system for testing the oncogenic potential of viruses used in human immunization.

DISCUSSION

A review of the most relevant data has shown that precise information concerning the possible risk of multiple immunizations is unavailable. There have been no studies in experimental animals or in man that analyzed the effect of immunization with several antigens given simultaneously. It is conceivable that this type of immunization involves a complex reaction affecting the entire regulatory immune system. The long-term effects of immunization with polysaccharide

antigens are also unknown. The review of the literature and the reports to the Committee by Drs. Thomas J. Mason and Robert N. Hoover (National Cancer Institute) and Dr. Michael Stek (Navy) indicate that the most probable complication of multiple immunizations is vasculitis of the serum-sickness type or Landry-Guillain-Barré syndrome. Admittedly, severe complications of multiple immunization, such as those reported here, must be exceedingly rare. However, some of the well-documented reports have indicated that persons exposed to multiple vaccines are occasionally exposed to the risk of serum sickness or of polyneuritis. Therefore, systematic studies with modern techniques that can detect initial signs of glomerular disease or the development of circulating immune complexes or autoantibodies are justified. The possibility of lymphoma, due either to excessive antigenic stimulation or to the oncogenic potential of particular viruses, also deserves attention.

The USAMRIID has complete immunization records on approximately 3,500 persons who worked at Fort Detrick and were subjected to intensive immunization. There are also frozen serum samples from many in this group. Drs. Mason and Hoover are conducting a study to determine how many can be located. Followup studies may be undertaken to include comparison of current and stored serum samples, provided that a significant number of vaccinees can be located.

Another potential study group consists of the workers who were engaged in the production of agents at Pine Bluff arsenal in Arkansas. Detailed immunization records are also available for these persons (William R. Beisel, personal communication).

In view of new technologic advances, it would be relatively simple to conduct prospective studies to determine the risk of immune-complex or autoimmune diseases. These studies would be important not only to the armed forces, which will presumably continue the use of multiple vaccinations to protect personnel, but also to the general biomedical scientific community, inasmuch as there is a paucity of information available on the subject.

RECOMMENDATIONS

The paucity of data in the literature concerning the undesirable effects of multiple immunizations, which often involve vaccines with multiple antigens, provides the Committee no basis to formulate a firm conclusion. No significant or adequate studies have been performed and reported in humans or experimental animals. Thus, the recommendations of the Committee are based on anecdotal reports, a few published reports, and data presented to the Committee by Drs. Mason, Hoover, and Stek. There is the prospect of a continued need to evaluate a wide variety of infectious agents in the armed forces and in other population groups, as well as the requirement to prepare and administer vaccines for some of them. Hence, the problem of long-term effects of repeated immunizations needs constant appraisal. The few leads from studies in Fort Detrick workers should be followed, and new methods of investigation, such as information about immunoglobulin allotypes, should be evaluated. The attempts of Drs. Mason and Hoover to contact former Fort Detrick and Pine Bluff personnel should be encouraged. Other retrospective and prospective studies are recommended, as described below. The rarity of adverse reactions (1 in 25,000 or even 1 in 5,000) makes it difficult to detect a significant abnormality. Results may signify only the absence of abnormal sequelae. There is always the likelihood that a larger study group will yield other findings. A study of 3,500 persons, or a substantial fraction thereof, will obviously be more significant than the smaller sample of 99 persons. Much more reliable information will accrue when newer test results are applied to the larger sample.

RETROSPECTIVE STUDIES

If available, autopsy records from personnel subjected to long-term immunization should be reviewed, with special attention to the occurrence of immune-complex or autoimmune diseases. The logistics of obtaining clinical data (and possibly serum samples) from living Army personnel immunized in the 1950s should be worked out by cooperation with local family physicians or faculty members of nearby schools of medicine or public health, once the personnel are traced.

PROSPECTIVE STUDIES

Systematic studies, including several measures of immune-complex or autoimmune diseases, should be undertaken in a suitable sample of Army personnel who have recently been immunized or who will be immunized repeatedly over long periods. Clinical and laboratory controls should help to determine whether any of these persons have or will have symptoms related to immune-complex or autoimmune disorders. Clinical records should be obtained periodically and interviews should be conducted by physicians. Special attention should be directed to blood-pressure measurement, urinalysis, and the measurement of serum creatinine and blood urea nitrogen. Laboratory tests should be performed before the beginning of immunization, 3 months after the beginning of immunization, and every year for 5 yr after the beginning of immunization. The tests that should be considered include assays for:

1. Humoral antibody response.
2. Rheumatoid factor.

3. Antigen-antibody complexes. Because various tests detect complexes with different biologic properties, at least three different assays should be used. The following three are all sensitive radioimmunoassays.

   a. Raji cell--Detects complexes near equivalence or in antibody excess and depend on activation of complement.

   b. A Clq solid phase--Depends on activation of complement and measures complexes formed in antigen excess.

   c. Monoclonal rheumatoid factor--Does not depend on activation of complement and detects small non-complement-fixing complexes.

4. Antinuclear antibodies (ANA) and other autoantibodies. If positive tests for ANA are obtained, the serum should be sent to a reference laboratory for further studies on the specificity of the antibody. Furthermore, serum from a patient with autoimmune disease should be tested by indirect immunofluorescence on fresh human tissues (kidney, nerve, central nervous system, etc.) for the presence of autoantibodies.

5. Serum-protein abnormalities.

   a. Serum-protein electrophoresis (agar-gel) and total immunoglobulin.

b. Quantitation of IgG, IgA, IgM, IgD, and IgE, using immune assay.

c. Immunoelectrophoresis or rocket immunoelectrophoresis.

d. Quantitation of total kappa-lambda, to detect imbalance.

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